centrifuging the cells again at 1,000 rpm,

5. Resuspending the cell pellets, transferring to a first Eppendorf tube in 1 ml of ice cold PBS,
6. Centrifuging at 2,000 rpm for 5 min at 4 °C removing the PBS processing the cell pellets according to the protein and DNA isolation steps comprising,
7. Preparing Buffer A: (cell lysis buffer), [20 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% NP-40, 10% glycerol, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 0.4 mM phenylmethylsulfonyl fluoride (PMSF), antipain (1 μg/ml), leupeptin (1 μg/ml)],
8. Preparing Buffer B: (extraction buffer without salt), [20 mM Hepes, pH 7.9, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 μg/ml), leupeptin (1 μg/ml)],
9. Preparing Buffer C: (extraction buffer with salt), [20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 μg/ml), leupeptin (1 μg/ml)],
10. Preparing Buffer D: (cytoplasmic extraction clarification buffer), [20 mM Hepes, pH 7.9, 400 mM NaCl, 0.2 mM EDTA, 40% glycerol, 1 mM DTT, 0.4 mM PMSF, antipain (1 μg/ml), leupeptin (1 μg/ml)],
11. Resuspending the cell pellets in 100-125 μl (2 pellet vol) of Buffer A,
12. Maintaining the resuspended cell pellets on ice for 10-15 min with occasional tapping,
13. Pelleting the nuclei by centrifuging at 2,000 rpm for 5 min at 4 °C,
14. Removing the cytoplasmic supernatant fraction to a second Eppendorf tube,
15. Quick freezing on dry ice in a -86 °C freezer and store for future use,
16. Washing the bottom nuclear fraction with 200-300 μl Buffer B to remove NP-40,
17. Centrifuging at 2,000 rpm for 5 min at 4 °C,
18. Resuspending the pelleted nuclei into 100-130 μl high salt Buffer C on ice for 45 min and mixing

periodically by tapping to extract the nuclear proteins,

19. Centrifuging the nuclear fraction in an Eppendorf centrifuge at 13,000 rpm for 15 min at 4 °C,
20. Removing the supernatents, aliquoted, in 25 µl and quick freezing on dry ice,
21. Storing at -86 °C,
22. Quick freezing the remaining pellet containing nucleic acids and other debris,
23. Storing at -86 °C,
24. Clarifying the cytoplasmic fraction by adding 1/3 vol of Buffer D to this fraction for 30 min at 4 °C to equilibrate the cytoplasmic proteins with NaCl,
25. Centrifuging at 13,000 rpm for 15 min.,
26. Removing and quick freezing the supernatants and storeing at -86 °C,
27. Performing DNA extraction and analysis comprising the steps of,
28. Thawing frozen cell pellets from on ice for 10 min,
29. Adding 100{1}µl of Buffer [0.1 % SDS, 10 mM Tris-HCl, pH 7.9, 10 mM EDTA, 10 mM NaCl] for 15 min,
30. Mixing using wide bore Eppendorf pipet tips,
31. Adding RNAase A for 2 h at 37 °C with gentle tapping every 30 min,
32. Adding proteinase K [200 {1}µg /ml] for 2 h at 37 °C with gentle tapping every 30 min,
33. Extracting with an equal volume of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) saturated phenol,
34. Removing the upper aqueous layer was removed to another tube,
35. Performing back extractions twice with 50 µl of TE,
36. Collecting the DNA solutions in a fresh tube,

37. Adding an equal volume of phenol/chloroform (50:50) mixture,

38. Mixing by inverting repeatedly,

39. Centrifuging at 3,000 rpm for 10 min,

40. Removing the upper aqueous phase,

41. Extracting with an equal volume of chloroform/isoamyl (96:4) alcohol,

42. Removing the upper aqueous phase containing the DNA and precipitating using 0.5 M NaCl and 3 vol of ice cold ethanol at -20 °C,

43. Cenrifuging the samples at 13,000 rpm for 30 min,

44. Air drying the DNA pellets,

45. Dissolving DNA in 300 µl of 0.1X TE at 37 °C for 4-6 h,

46. Digesting ten {l}µg of the high molecular weight DNA from each sample for 4 h with 30 units of restriction enzymes,

47. Electrophoresing on a 0.8% TAE (0.40 M Tris-acetate, 1 mM EDTA) agarose gel,

48. Staining the DNA gel with ethidium bromide,

49. Photographing with UV light,

50. Radiolabeling of the sequence specific oligonucleotides comprising the steps of,

51. Synthesizing single stranded oligonucleotides on a DNA synthesizer and annealing with the complimentary strand by combining 4 µg of both strands in a tube with total volume of 30 µl of annealing buffer (5 mM NaCl, 10 mM Tris-HCl and 0. 2 mM EDTA),

52. boiling the tubes for 5 min and slowly cooling to room temperature for 6 h,

53. Heating the tubes to 55 °C for 5 min and then cooling on ice for 10 min,

54. Quantitating 4 µl aliquot of the annealed oligos at 260 λ and storing the remainder at -20 °C until radiolabeled,

55. Preparing probes by radiolabeling 200 ng of annealed oligonucleotide in 15 µl of total volume containing 50 µCi of [γ-^{32}P]ATP (6,000 Ci/mmol), 20 Units of T4 polynucleotide kinase, and 1.5 µl 10X T4 polynucleotide kinase buffer for 1 h at 37 °C,

56. Filling in the 5' over-hang ends with 5 Units of Klenow with 3.0 µl of 10X Klenow buffer and 0.15 mM each of dATP, dCTP, dGTP, and dTTP for 40 min at 37 °C in a reaction volume of 30 µl,

57. Increasing the volume to 1 ml with sterile TE with 200 mM NaCl, pH 8.0, and the labeled oligonucleotides were purified on a NACS Prepac column, to separate the unincorporated nucleotides,

58. Precipating the labeled, purified oligonucleotides overnight with 3 vol absolute ethanol at -20 °C,

59. Centrifuging at 13,000 rpm for 1 h and then vacuum drying,

60. Resuspending the labeled oligonucleotide probes in 100 µl of sterile 0.1X TE buffer and storing at -20 °C,

61. Transcriptional protein and sequence specific DNA binding analysis by Electrophoretic mobility shift assay,

62. Incubating 5 µg of nuclear or cytoplasmic extract, for each reaction, with 0.2-0.3 ng of [γ-^{32}P]ATP labeled oligonucleotide probe containing either NF-κB sequence (5'-gatccGGGACTTTCCGCTGGGGACTTTCCG-3') or an AP-1 consensus sequence including the PMA responsive element indicated in bold (5'-gatccG**TGACTCA**GCGCG-3'),

63. Adding 3 µg of poly(dI-dC):poly(dI-dC) as a non-specific competitor and incubating with the nuclear extracts for 10 min prior to the addition of the radiolabeled probe,

64. Adding antibodies against p65, p50, c-*Fos* or c-*Jun* to the respective binding reactions for supershift assays and incubating at room temperature for 1.5 h, prior to probing with [γ-^{32}P]ATP labeled oligonucleotide for an additional 25 min at room temperature,

65. Separating the bound complexes on either a 5% ,for supershift assays, or 6% ,for analytical purposes, acrylamide/bis (30:1 ratio) native gel as required and runing at 200v for 3.5 h with 0.25X TBE (0.02 M Tris-borate, 0.5 mM EDTA) as running buffer at room temperature,

66. Vacuum drying the gel with heat at 80 ^{o}C and exposing them to Kodak X-OMAT film for 3-12 hours,

67. Analyzing the bound and free DNA protein complexes {.} whereby simultanous isolation of biologically active transcription factors and DNA is accomplished.